

# Studies in Closed Ecological Systems: Biosphere in a Bottle

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## Abstract

Small closed ecological chambers are an efficient model for developing principles of bioregenerative systems. Knowing these principles can enhance long distance space travel and remote human habitation. Closed systems were constructed in the laboratory using tissue culture flasks and one-liter glass bottles. All nutrients, micro algae and aquatic macro-invertebrates were added approximately one week before sealing. Viability of the closed system was judged by continued survival and activity of macro-invertebrates. Teams studied varying light intensity, nutrient concentration, temperature and varying air space. We found that *Tigriopus californicus* (a salt water copepod detritus feeder) populations maintained a steady abundance at high intensity light, increased quickly at moderate light and declined quickly at low light. *Daphnia magna* (a fresh water zooplankton) population increased more rapidly at high temperature. Within the levels of nutrient concentration we tested, *T. californicus* population growth increased with more algal nutrients. While trying to test the relationship between air space and viability, we observed that when refuges were present for algae and macro-invertebrates, populations outlived those without refuge space. Studies are continuing.

## Introduction

Attempting to reconstruct nature in a bottle appears to be a formidable task. Nature is very complex with organisms at all levels, from macro to micro, interacting with each other and changing abiotic factors. However, developing closed environments will benefit our studies of life and the universe. Small materially closed microcosms have been studied since the late 1960's (Folsome, 1986). Since then several books, journal articles and PhD dissertations have been published about closed microcosms. We have taken a small piece of nature to produce a small, simple closed systems to test the systems responses and tolerance to varying initial conditions (nutrient, air:water volumes) and energy inputs. For the purpose of this study, we define

a successful ecosystem as one in which all the macro-zooplankton populations persist. The survival of the organisms serves as a biomarker that oxygen is available and chemical conditions do not exceed their tolerance.

The Earth's biosphere is made up of interacting ecosystems, each characterized by abiotic and biotic factors. Abiotic factors, such as temperature and light, influence the distribution of biotic factors and may in turn influence the abiotic factors of other ecosystems. For example, local wind and water currents can eventually influence global atmospheric and oceanic circulation patterns. It is difficult to predict what constraint may limit the growth of organisms, or cause a decline in population. It is even harder to determine which species will dominate due to the availability of certain resources.

The four most important chemical elements for living organisms are hydrogen, oxygen, carbon and nitrogen (Adey and Loveland 1998). Many other elements are necessary for sustained life, but in much smaller quantities. Life is constrained when any needed element is in short supply. Alternately, a needed element may be abundant, but in an unusable form. Free nitrogen ( $N_2$ ) is plentiful in the atmosphere, but is often a limiting factor for plant growth in the absence of nitrogen fixing bacteria such as Cyanobacteria. Also, a needed element may be missing entirely in a particular ecosystem, but its absence overlooked for some time, because it is needed in such small quantities.

Some ecosystems on earth seem to have obvious abiotic constraints. In the Gobi Desert, we expect water to be the primary constraint on biomass growth. Other ecosystems have constraints that are not as obvious. This is the case in the equatorial Pacific, which is a barren ocean that blooms profusely when iron levels in an available form are boosted by only a few parts per billion (Martin et. al. 1994; Rue & Bruland, 1997). Subtle constraints may lead to problems that appear to be caused by more obvious constraints. For example a lack of magnesium would inhibit photosynthesis and may lead to a lack of  $O_2$ ; the lack of  $O_2$  may appear to be the primary constraint when in fact it is secondary.

Density dependent growth refers to the constraints that are put on a population as it expands relative to its ecosystem. If it grows too quickly, the population can

deplete the necessary nutrients it needs to grow and survive. The rapid increase may be followed by death of part or all of the population. In a closed system, the growth of the population must be controlled so that production of nutrients can keep up with the growing population's increasing demand. There are several ways to do this in a small system. Temperature is one way. Low temperature slows the metabolism of many animals. With a slowed metabolism, the animal will eat less, thus grow and reproduce less. Temperature is not as much of a restraining factor for plants. If plants are able to grow and reproduce at a normal level, both the food for the animals and the available  $O_2$  will increase, hopefully at a rate equal to that of the consumption by the animal.

In an open ecosystem, organisms can migrate between ecosystems. Plant seeds / spores may be dispersed far from the parent plant by animals, ocean currents or wind. Both biotic and abiotic factors can buffer the ecosystem from some extremes by migrating to neighboring ecosystems in this way. On Earth, with its complex ecosystem and diverse terrain, it is often possible to compensate for material deficiency at one locale, simply by moving to a new one. For example, humans may have populated North America as hunters following one of their constraints, large herd animals, across the Bering land bridge. Also,  $O_2$  and  $CO_2$  can diffuse between the air and the aquatic community compensating for imbalances.

In a closed system, nutrients may be limited because they are not cycled through the system fast enough and/or it is not possible to compensate for deficiencies by migration. In closed ecosystems, outside influences are eliminated. Therefore closed ecosystems test the adequacy of the initial organism and nutrient supply. The challenge in finding appropriate initial conditions and radiant energy inputs for a closed system lies in the constraints of these factors. Once the system is sealed, we can observe the interplay of the organisms.

We have defined the success of our model ecosystems as the persistence of macro-invertebrate populations. There may be several periods of population fluctuation. In some systems, an initial species of the system may be reduced or go extinct as it is followed by a subsequent species. In this way an early dominant species may be replaced by another species, previously rare, that becomes dominant. This is an example of succession. In primary succession, the initial colonizing species often

prepares a new ecosystem condition with necessary nutrients for a secondary species that will replace the primary species. A terrestrial example of this is a nitrogen fixing plant that colonizes a barren area after a major disturbance. The nitrogen fixing plant will build up the nitrogen store in the soil, providing the secondary species the necessary nutrients to populate the area, after which the secondary species may crowd out the earlier dominant species.

Balanced ecosystems do not always start out as balanced systems. Given the correct initial conditions, however, they may evolve to a balanced state. In closed ecosystems, the primary species may be an alga that grows quickly, providing an initial food source for the invertebrate while a secondary, hardier algal species accumulates. In a second example, the primary species may be an invertebrate that grows and reproduces quickly, adding to the carbon storage by molt and/or skeletons while a second, hardier but slower growing species gets started. Since there is no immigration into the system, it is difficult to find the right balance of organisms to start a system. In a closed system with sustainable population fluctuations, populations rarely grow as large as they would in an open system. The population size is dependent on the available nutrients. Thus the population is density dependent. An example of this was found in an experiment on sewage oxidation in 1959. An in vitro system was made in an attempt to mimic an oxidation pond. In the synthetic system, a succession began with a bacterial bloom, followed by an algal bloom of *Chlorella*, and then followed by an increase in invertebrate population. The climax was observed to be similar to the population from the original pond. One of the links that allowed this succession to take place was the excretion by the bacteria of thiamine, a vitamin necessary for growth of the strain of *Chlorella* (Byers & Odum 1993).

We can monitor small, closed ecosystems more fully than large open systems, because the complexity is reduced, allowing for easier measurements and experimental controls. If we can learn which initial conditions and energy inputs allow the small system to sustain macro-invertebrate populations, we can scale up these small ecosystem models to predict the requirements for sustainability in larger ecosystems.

## **Experimental Background**

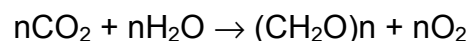
Small, simple closed microcosms are a means of monitoring short-term pressures on an ecological system. Claire Folsome (1986) pioneered work on closed ecological systems in the late 1960's, and many journal articles, books (Giese, 1978; Byers & Odum, 1993), and PhD dissertations have followed. Despite this, small closed ecological systems are rarely used as serious research tools, but marketed to the public as curiosities. One reason for not using them for research may be expense.

Biosphere II and NASA's Closed Ecological (or Engineering) Life Support Systems (CELSS) are very expensive. Small microcosms developed in tissue culture flasks are inexpensive, enabling us to study simple questions, while providing the opportunity for several replicates and controls.

There are two major controversies about small, synthesized ecosystems or microcosms. The first is that ecosystems are too complex for human synthesis. It is believed that there are too many interactions between abiotic and biotic factors for a human to produce a working ecosystem. The other controversy is that in order to control an ecosystem it must be simple - i.e. a monoculture or single species gardening approach. These two controversies need to be considered and accommodated when building a closed system that will be used to study the interaction of species in an ecosystem and to test the effects of constraints (i.e. limited nutrients, light energy or toxicity) or to allow for human habitation in barren habitats.

"The process occurring in microcosms are the same as those found in ecosystems, but they are simplified since the system is closed and isolated" (Byers & Odum, 1993). Knowing the fundamentals of closed system constraints will be necessary for human habitation of such systems in the context of space travel and colonization.

One hypothesis is that an important constraint on a closed ecological system is oxygen, since it is almost entirely biologically produced. Plants and some bacteria produce oxygen by photosynthesis. Photosynthesis is the process by which organisms use light energy to break apart water and fix carbon as glucose. The equation for photosynthesis is:



Respiration is the same process in reverse, where glucose is broken apart and combined with  $O_2$  to get energy,  $CO_2$  and  $H_2O$ . Especially in small systems, periods of low levels of  $O_2$  or no  $O_2$  can kill some organisms very quickly. There are several ways to buffer against this catastrophe. Increasing the plant population will increase the ability of the system to maintain  $O_2$  levels. Likewise decreasing the animal's metabolism, and thus respiration, will decrease the amount of  $O_2$  used. Adding pH buffers to the system via air space or salts in the water will protect against extreme pH changes and add a place for  $O_2$  to be stored. Another unexplored option is to use species that are anoxia tolerant. These species can produce spores or eggs that can tolerate anoxic conditions for long periods of time, allowing the system to recharge its oxygen stores. Some species may even be active in anoxic conditions. Nematodes are an example of this.

If a crew of astronauts runs out of oxidative potential while on the long trip to Mars, they cannot stick their heads out the window to catch their breath. Once on Mars, they will continue to need oxygen. In addition to the long-term balance that must be obtained, short-term constraints must also be monitored. It does us no good to get to Mars with a full load of oxygen, if all of the Astronauts are dead from asphyxiation because six weeks into the trip an unexpected bacterial bloom used up all of the oxygen for two hours. Plants will probably produce this oxygen, but if there is a shortage of nitrogen, an element that is in short supply on Mars compared to Earth, then the plants cannot grow and will not produce oxygen. Furthermore, the nitrogen must be in a usable form, and so nitrogen-fixing bacteria may be needed. But Cyanobacteria depend on complex water chemistry, which often involves hundreds or thousands of species. It will be much easier to get a general grasp of these systems, than to engineer a complex network of dependencies from individual biochemical reactions.

We ran a series of pilot studies to investigate the range of conditions for constraints for  $O_2 / CO_2$  equilibrium in closed aquatic systems. The biomarker we used to determine anoxia in systems was obtained by quantifying populations of macro-invertebrates determined to be tolerant of anoxic conditions for times in excess of 24 hours (fig. 6). This allowed us to experimentally examine the effects of a number of

constraints on the O<sub>2</sub> / CO<sub>2</sub> equilibrium of the enclosed system. Our experiments attempted to develop isolated ecosystems that functioned normally for reasonably extended periods under a series of pressures. "The general tactic [has been] to enclose aliquants of ecosystems; to observe how and, if possible, deduce why the systems changed or failed; to modify conditions appropriately; and to experiment again" (Macguire 1980). Any system that contains living macro-invertebrates is assumed to have not had chronic anoxia.

### **Approach**

All systems were constructed in glass or thick plastic containers and sealed with screw caps. All water, chemicals, and algae were added two to seven days before invertebrates to allow for any unexpected organic material oxidation. Systems were sealed on the same day the macro-invertebrates were added. Unless mentioned, all systems were kept in normal room temperature (~21° C) away from direct sun light, room temperature or light controls.

The three types of micro algae used for freshwater were *Selenastrum*, *Chlamydomonas*, and *Ankistrodesmus* - all micro algae that serves as a food source for the freshwater invertebrates. The freshwater invertebrates that were used were *Daphnia magna* and / or *Ceriodaphnia*. T82, a freshwater algal media served as the source of micronutrients for the algae (table 1). Kent water (table 3) was used as the source for freshwater.

For salt water systems, the three types of micro algae used were *Nannochloropussis*, *Isochrysis*, and a green mixture - which served as a food source for the invertebrate *Tigriopsis californicus*. f/2, a marine algae media served as the source of micronutrients for the algae (table 2). Filtered, UV disinfected salt water was obtained through the Seattle Aquarium.

*Light Intensity Experiment:* Tissue culture flasks (65 ml) were filled with 60 ml seawater, 5% f/2 media, and 0.1 ml of each salt-water micro algae (*Nannochloropussis*, *Isochrysis*, and a green mixture). Seven days after filling the flasks, 6 *Tigriopsis californicus* were added to each flask and each flask was sealed. Each flask was slipped into a pocket constructed out of 50% light-blocking greenhouse shade cloth. Replicates of 3-4 were set up at four light intensities, created by putting different layers of shade cloth around each flask.

*Temperature Experiment:* Glass bottles (1 L) were filled with 800 ml Kent water, 200 ml T82 media, and some of each freshwater micro algae (*Ankistrodesmus* and *Scenedesmus*). Seven days after filling the flasks, 5 *Daphnia magna* were added to each bottle and the bottles were sealed. Each bottle was then placed in a water bath at varying temperatures (25° C, 15° C, and 20° C) and control bottles were placed at room

temperature (approx. 21° C). The water baths were kept at a constant temp by using an aquarium heater for heat or running cold water to cool. Fluorescent lights were mounted above systems to provide light and were on a 12 hour on / 12 hour off timer.

**Nutrient Experiment:** Tissue culture flasks (65 ml) were filled to the rim with varying concentrations of seawater / f/2 media (2%, 10% and 20%), and 0.1 ml of each salt water micro algae (*Nannochloropussis*, *Isochrysis*, and a green mixture). Two days after filling the flasks, 6 *Tigriopsis californicus* were added to each flask and each flask was sealed.

**Air Space Experiment:** 65 ml tissue culture flasks were filled with 30 ml Kent water, 10 ml T82 media, and 0.1 ml of each of the freshwater micro algae (*Selenastrum*, *Ankistrodesmus*, and *Chlamydomonas*). A 48-hour toxicity test was performed to determine possible toxic effects of the styrofoam on the *Daphnia*. There were no apparent adverse effects, so the experiment set-up proceeded. Seven days after filling the flasks, 6 *Daphnia magna* and 6 *Ceriodaphnia* were added to each flask. Styrofoam was then added to each flask at varying volumes (20 ml, 10 ml, and 5 ml; measured by water displacement) to displace air in the flask. 14 ml of T82 media was then added to each flask to raise the level of liquid in the flask with the highest volume of styrofoam to the rim (to displace any remaining air) and each flask was sealed.

**Salinity Experiment:** Tissue culture flasks (1000 ml) were filled with varying concentrations of salt water (6.25%, 12.5%, and 25%), Kent water, and 25% T82 to which 25 ml of *Selanastrum* was added. Five days after filling, five *Daphnia magna* were added to each flask and each flask was sealed.

## **Results:**

*Tigriopsis californicus* populations grew at a faster rate when systems were in environments with intermediate light intensities (13.7 to 23.4  $\mu$ Einsteins). At the highest light intensities the population persisted but did not increase. At the lowest light level, 7.7  $\mu$ Einsteins, the population was low, but started increasing at day 30 and by the end of the experiment equaled the highest density. No populations went extinct during the 40-day experiment (fig 1).

*T. californicus* populations were the highest at the end of the 42-day experiment in flasks with intermediate (10%) concentrations of T82 algae growth media. Populations in flasks with higher concentrations had the highest daily average population throughout the course of the experiment, and had the lowest final population. No populations went extinct during the course of the experiment (fig 2).



*Daphnia magna* grew at the fastest rate in bottles kept at the highest temperature (25° C) and also declined the fastest. Populations kept at room temperature kept a constant population size the longest. Populations in the highest temperature (25° C) were extinct within 49 days and the populations kept at the lowest temperature (15° C) were close to extinction (fig, 3).

*D. magna* in flasks with no free air space declined at a faster rate, but lasted longer than those in flasks with air space (except control). All flasks went extinct by day 17, except one control flask and one flask with no air space - both that went extinct by day 20. Populations grew in all flasks, except those without free air space (fig. 4).

### **Conclusion:**

We produced simple model systems for ecological studies by enclosing micro algae and invertebrates in closed flasks. These model systems are similar to *Arabidopsis* for botany studies, *Drosophila* for zoology studies, Zebra fish for fisheries studies, and yeast for genetics studies. Oxygen levels were monitored by observation of invertebrate populations that are sensitive to anoxia. Various constraints (light, temperature, algal nutrient concentration, salinity and air space) were placed on the systems to quantify the range of the sensitivity to these pressures.

Results showed that a difference in our variables led to different population patterns. Medium light intensities (13.7-23.4 micro Einsteins), 10% algal growth media, intermediate temperature (20-21°C), and 6.25% salinity were found to support higher populations of invertebrates than variables at other levels. The addition of seawater was to supply trace levels of elements that might be lacking in the chemically defined algal medium (T82) or the Kent water. The salt water also served to test the osmotic tolerance of the organisms.

Further experiments need to be done to assess the effects of complexity of species assemblage, complexity of environment (refuges) and anaerobic microzones.

Our studies are beginning to give us an idea of the time scale of these types of systems as well as the kinds of factors that affect their population patterns. (Our salinity experiment showed a definite effect, whereas our variation in air space did not have a conclusive effect). Our next step will be to define base systems to work with. Using

variations of these systems, we will build a mathematical model. We will continue to work with variation in air space (gas buffer), nutrient concentration, light intensity, temperature and toxicity (salinity). In addition, we plan to look at genetic variation (of a single species), size variation of the system, a harvesting situation where the system is expected to produce a needed commodity (food, O<sub>2</sub>) and complexity of space within the closed system (refuges).

We will also develop assays for better determining the health of our systems. We will refine our understanding of the needs of our chosen indicator species (*Daphnia magna*). We will start monitoring pH and O<sub>2</sub> concentrations extracted from our systems. We will also attempt to analyze the long-term viability of some of our systems using energy in equals energy out model.

Ecosystems have been modeled mathematically for some time. In the best case of a simple system, the mathematics are both intricate and, over long periods, inaccurate. One aspect which all of these have in common is the input of the light and the output of heat. Since these are the only input and output in our systems, they may give us an overall view of what the system will do down the road. If input and output are not equal, then something is building up or is being depleted within the system. This will most likely lead to succession of species as conditions change. Our system model will look something like figure 7 with  $E_{in} = E_{out}$  being our goal. The diagram was drawn using the system developed by Byers and Odum (1993).

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**Table 1**

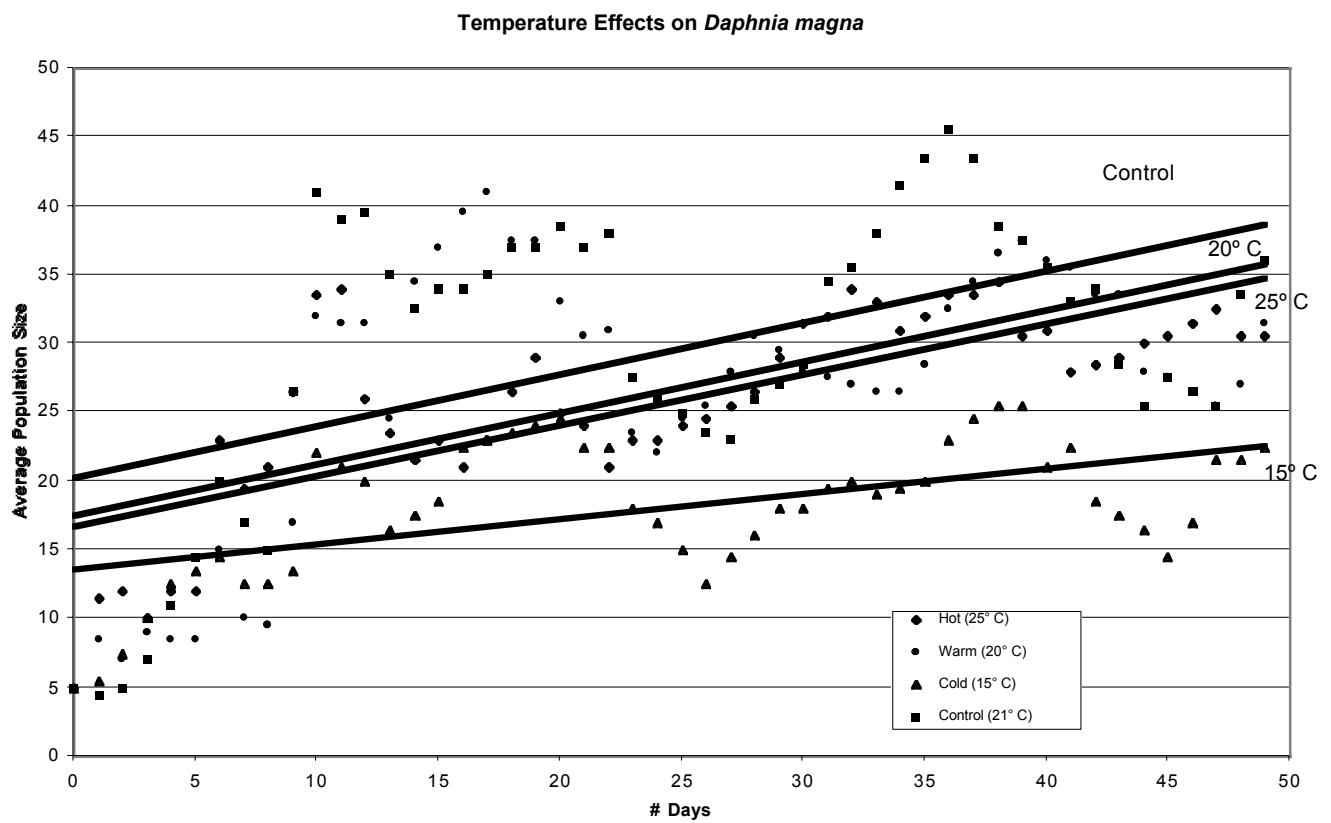
<b>Freshwater Algal Media (T82)</b>	<b>(Taub, 1993)</b>	
Compound	Element	
NaNO <sub>3</sub>	N	7.0 mg/L
MgSO <sub>4</sub> • 7H <sub>2</sub> O	Mg	2.43 mg/L
KH <sub>2</sub> PO <sub>4</sub>	P	1.23 mg/L
NaOH	Na	2.27 mg/L
CaCl <sub>2</sub> • 2H <sub>2</sub> O	Ca	40.0 mg/L
NaCl	Na	34.5 mg/L
Al <sub>2</sub> (SO <sub>4</sub> ) <sub>3</sub> • 18H <sub>2</sub> O	Al	0.26 mg/L
Na <sub>2</sub> SiO <sub>3</sub> • 9H <sub>2</sub> O	Na	36.8 mg/L
	Si	22.4 mg/L
FeSO <sub>4</sub> • 7H <sub>2</sub> O	Fe	0.0625 mg/L
EDTA	EDTA	0.4145 mg/L
H <sub>3</sub> BO <sub>3</sub>	B	0.008 mg/L
ZnSO <sub>4</sub> • 7H <sub>2</sub> O	Zn	0.0015 mg/L
MnCl <sub>2</sub> • 4H <sub>2</sub> O	Mn	0.0135 mg/L
Na <sub>2</sub> MoO <sub>4</sub> • 5H <sub>2</sub> O	Mo	0.0024 mg/L
CuSO <sub>4</sub> • 5H <sub>2</sub> O	Cu	0.00032 mg/L
Co(NO <sub>3</sub> ) <sub>2</sub> • 6H <sub>2</sub> O	Co	0.00015 mg/L

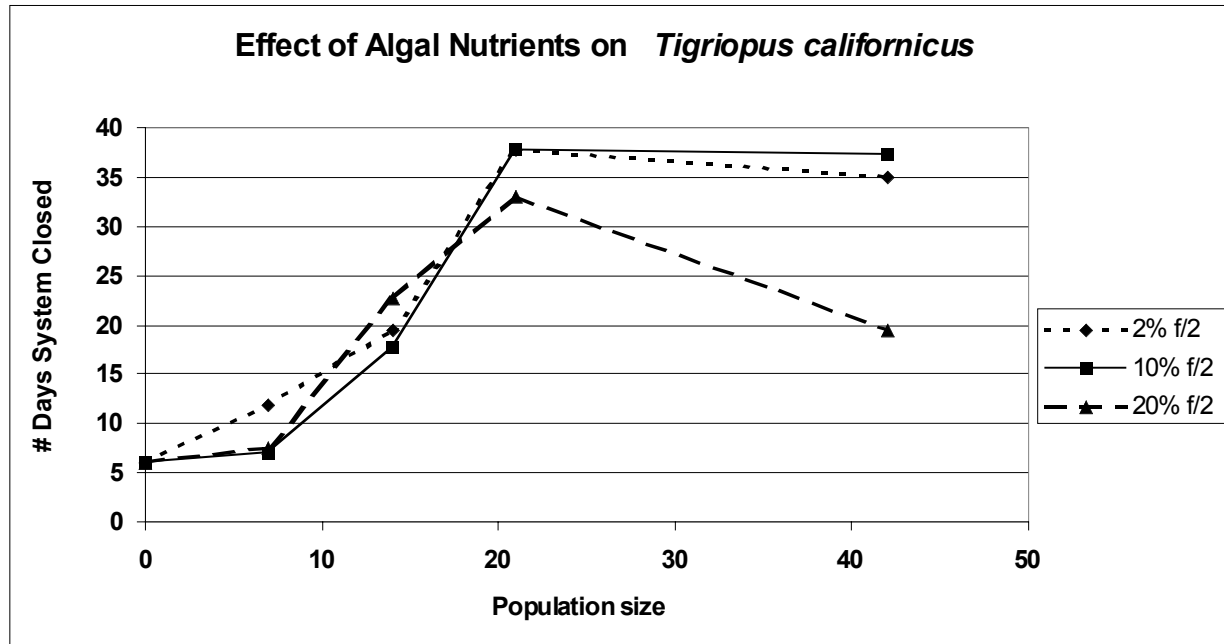
**Table 2**

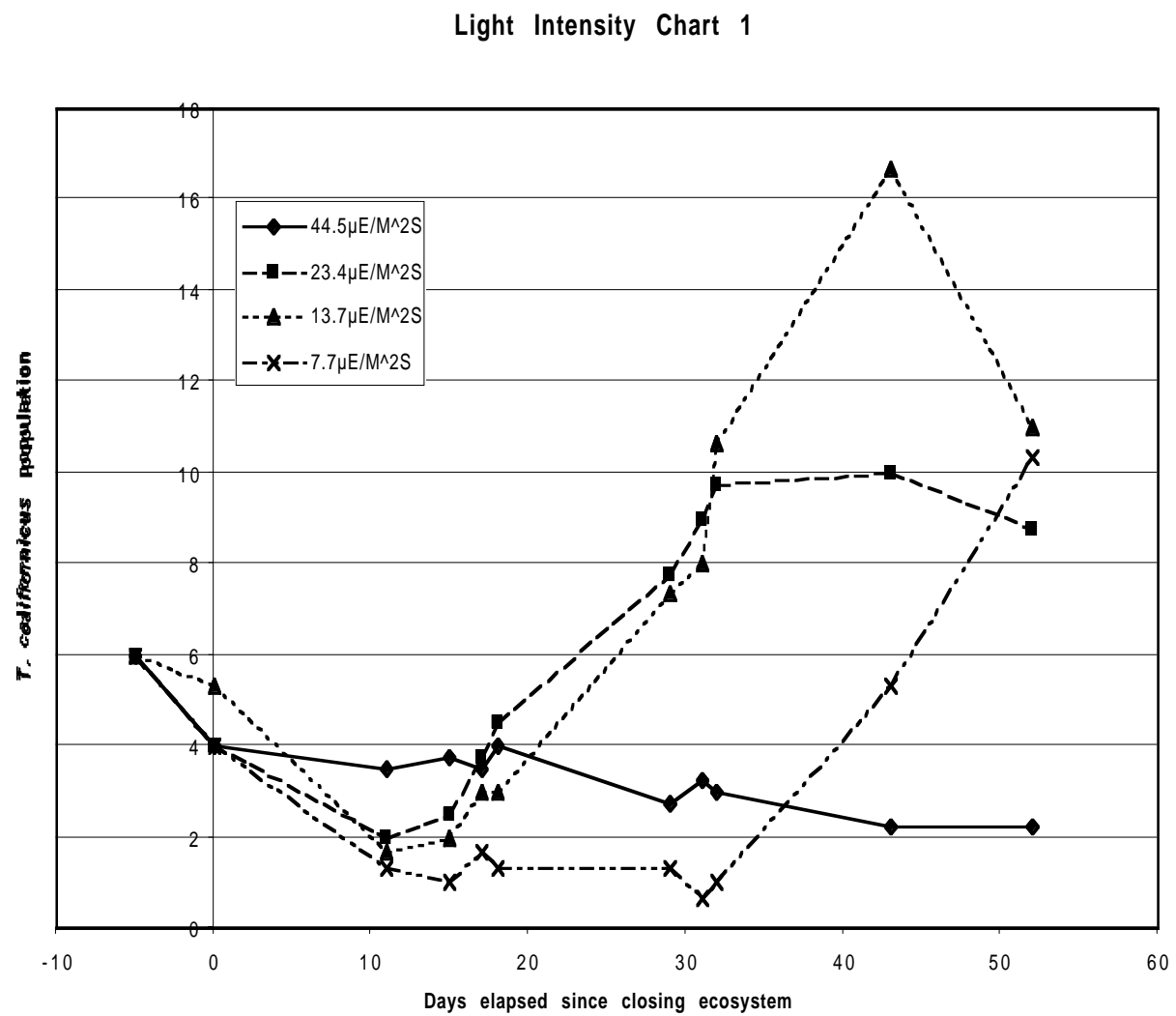
<b>Salt Water Algal Medium (f/2)</b>	<b>(McLachlin, 1973)</b>
NaNO <sub>3</sub>	0.075 g/L
NaH <sub>2</sub> PO <sub>4</sub> • H <sub>2</sub> O	0.005 g/L
CuSO <sub>4</sub> • 5H <sub>2</sub> O	0.25 ml/L
ZnSO <sub>4</sub> • 7H <sub>2</sub> O	0.25 ml/L
CoCl <sub>2</sub> • 6H <sub>2</sub> O	0.25 ml/L
MnCl <sub>2</sub> • 4H <sub>2</sub> O	0.25 ml/L
Na <sub>2</sub> MoO <sub>4</sub> • 2H <sub>2</sub> O	0.25 ml/L
O3 Stock A	0.76 ml/L
f/2 vitamins	0.5 ml/L
TRIS	5.0 ml/L

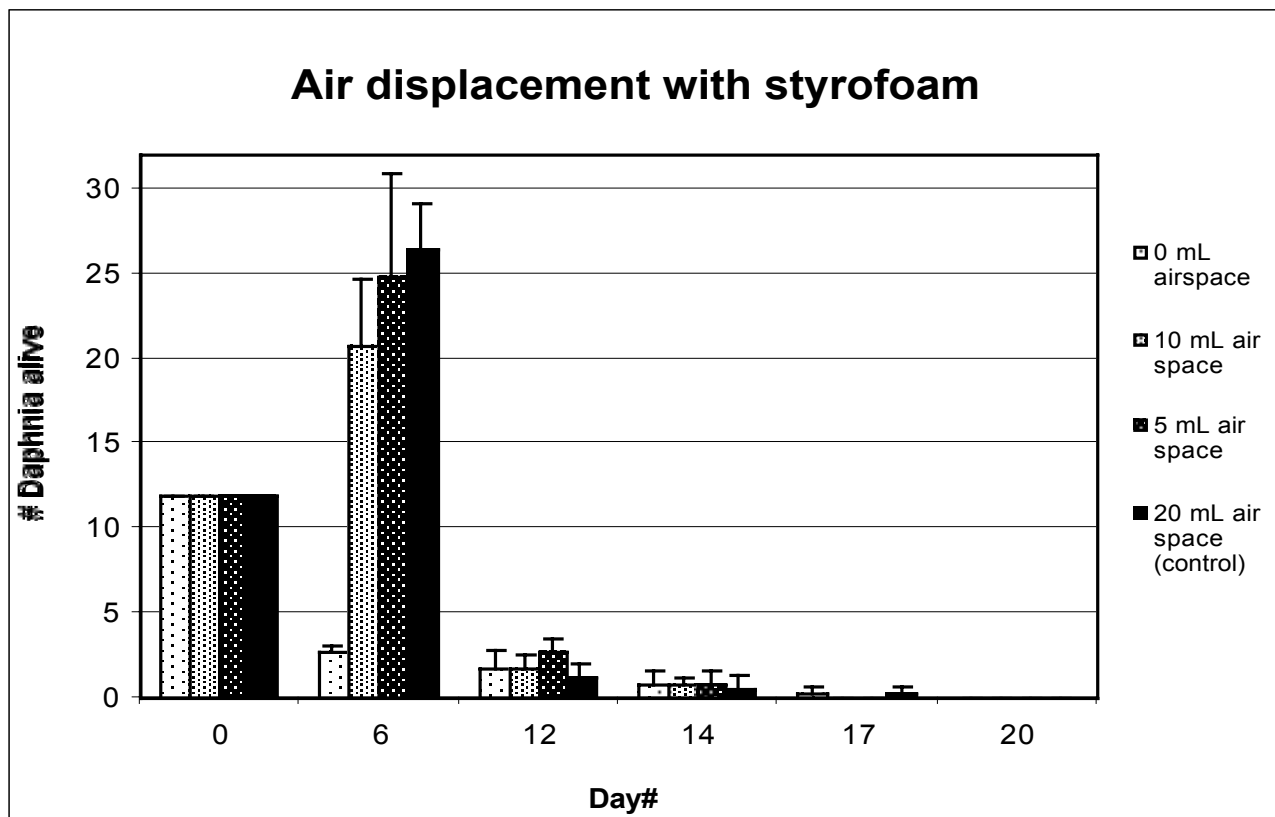
**Table 3**

<b>Kent Water</b>	<b>(Kent Marine, Marietta, GA)</b>
A combination of carbonates, sulfates and chlorides of sodium, magnesium, calcium and potassium with all necessary minor and trace metals necessary for cichlid fish. Contains no phosphates, nitrates or organics.	

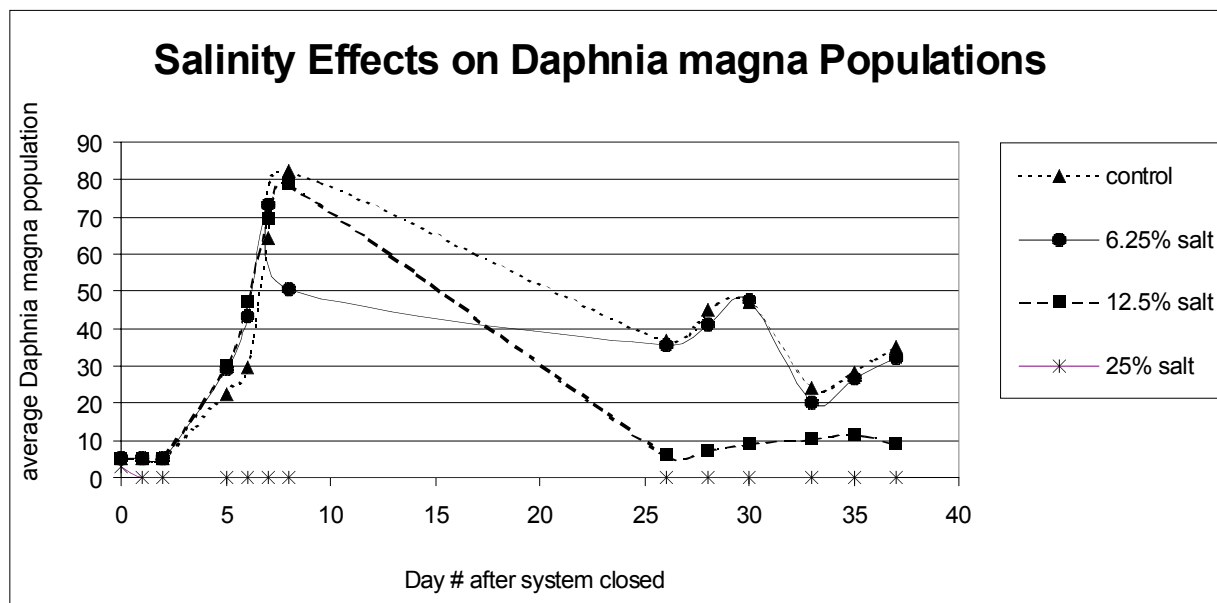
**Figure 1:**

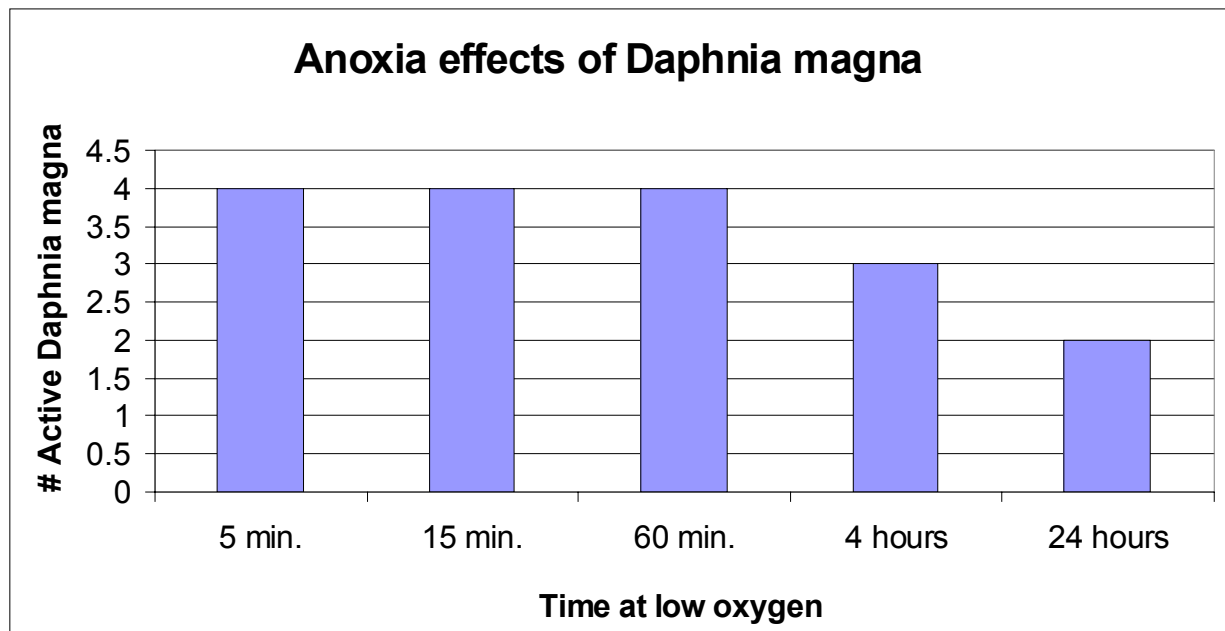
**Figure 2:**

**Figure 3:**

**Figure 4:**



**Figure 5:**

**Figure 6:**

**Figure 7: Energy Flow Diagram**